

A NOVEL APPROACH TO THE STUDY OF MAMMALIAN CELL-MEMBRANES USING DEUTERIUM NMR

Gösta ARVIDSON

University of Lund, Department of Physiological Chemistry, P.O. Box 750, S-220 07 Lund 7, Sweden

and

Göran LINDBLOM and Torbjörn DRAKENBERG

*The Lund Institute of Technology, Division of Physical Chemistry 2,
Chemical Center, P.O. Box 740, S-220 07 Lund 7, Sweden*

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1. Introduction

Several recent studies using different physical techniques [1,2] indicate that the lipids of cell-membranes are ordered in a bilayer structure. However, very little is known at present concerning the details of the interactions between different membrane components e.g. lipid-protein, lipid-lipid and protein-protein interactions. To investigate such interactions at the molecular level, spectroscopic methods have to be used. Magnetic resonance techniques (ESR and NMR) have been shown to be very potent tools for studies of both dynamic and structural properties of membranes [3,4]. Most of these studies have been performed on phospholipid model membranes [5,6]. Furthermore, some criticism has been raised against the ESR spin label method used in many studies of natural membranes, since the perturbation of the system studied with this method cannot be neglected [5,7].

We have previously shown that deuterium NMR studies on model membrane systems can be used to investigate conformational changes of the choline group of synthetic lecithins [8]. In the present report this method has been adapted to the study of rat-liver cell-membranes where the lecithin and sphingomyelin is labeled *in vivo* with [Me-²H] choline. This technique causes no distortion of the membrane and permits

detailed investigations on the lipid structure and lipid-protein interactions.

2. Experimental

[Me-²H] Choline was prepared according to du Vigneaud et al. [9] by adding C²H₃I (Ciba) to ice-cooled ethanolamine (British Drug House). The reaction mixture was left at room temperature with occasional shaking for two days. The content of the reaction vessel was then dissolved in water and the choline was purified by ion exchange chromatography on AG 50W-X-8, 200-400 mesh (Bio-Rad).

To 300 g of a choline-deficient diet (Nutritional Biochemicals) was added 6 mmol of the deuterated choline. This diet was given during 6 days to 6 male Sprague-Dawley rats, weighing 90–95 g. The rats were then killed and the livers homogenized in ice-cold 0.25 M sucrose–0.01 M Tris (pH 8.0) – 1 mM EDTA. A Teflon-glass homogenizer was used and the final dilution in the homogenate was 1 g, wet weight, of liver per 10 ml sucrose–Tris–EDTA. The homogenate was centrifuged at 900 *g* for 10 min and the resulting supernatant was then centrifuged at 15 700 *g* for 10 min in a Servall centrifuge. The mitochondrial pellet was resuspended in sucrose–Tris–EDTA and recentri-

fused. An aliquot of the sedimented mitochondria was directly transferred to a small test-tube for NMR-spectroscopy and the remainder was extracted with chloroform/methanol 2:1 (v/v). The total lipids of this extract were dried in vacuo, transferred to an NMR-tube and mixed with water. The concentration of water was 30% (v/w). Microsomes were isolated by centrifugation of the first 15 700 g - supernatant for 30 min at 105 000 g in an MSE 65 Superspeed Ultra-centrifuge. The microsomal pellet was treated in the same way as the mitochondria.

Deuterium NMR measurements were performed at 15.4 MHz with a Varian XL-100 spectrometer operating in the Fourier-transform mode. The pulse width was 100 μ s. A spectral width of 5 kHz and an acquisition time of 0.1 s were used. Mostly 400 free induction decays were accumulated and Fourier-transformed with a Varian 620 computer.

3. Results and discussion

Fig.1 shows the ^2H NMR spectrum for a non-oriented lamellar liquid crystalline sample prepared by mixing water and the lipids extracted from [Me- ^2H] choline-labeled rat liver microsomes. The spectral shape is a superposition of NMR signals with different quadrupole splittings due to a random distribution of bilayer orientations with respect to the external magnetic field. The quadrupole splitting $\Delta(\theta)$ for a macroscopically aligned sample i.e. where all the bilayers

have the same direction relative the applied magnetic field is given by [10] :

$$\Delta(\theta) = |\nu_Q S (3 \cos^2 \theta_{LD} - 1)|$$

where ν_Q is the quadrupole coupling constant, S the order parameter characterizing the degree of orientation of the electric field gradient around the normal to the bilayer, often called the director. θ_{LD} is the angle between the director and the applied magnetic field vector. For a nonoriented sample all orientations of the director are equally probable and the distance between the two absorption maxima observed in the NMR spectrum (see fig.1) corresponds to that for $\theta_{LD} = 90^\circ$ of a macroscopically aligned sample. We call this distance the quadrupole splitting, Δ_p , which consequently is given by the expression:

$$\Delta_p = |\nu_Q S|$$

The order parameter, S, may thus be calculated if the quadrupole coupling constant, ν_Q , is known. This is often the case when the quadrupole interaction is of intramolecular origin as e.g. in the case for a C- ^2H -bond, where the quadrupole coupling constant may be set to be 170 kHz as has been determined for paraffins [11]. In fig.2 is shown a deuterium NMR spectrum from the deuterated choline groups of rat liver mitochondrial membranes. The observation of a quadrupole splitting of these membranes implies that the lipids are oriented in an anisotropic way, and is compatible

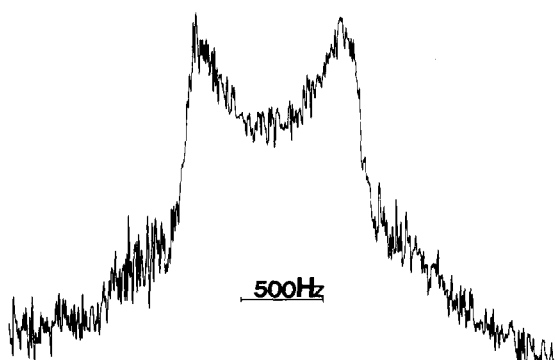


Fig.1. ^2H FT-NMR spectrum of a lamellar liquid crystalline sample of total mitochondrial lipids labeled with [Me- ^2H] choline. The water content of the sample was 30% (v/w) and the temperature about 28°C.

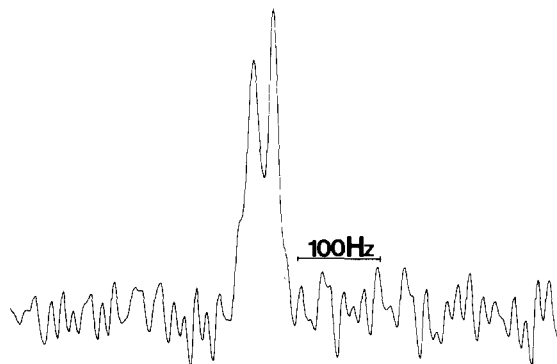


Fig.2. ^2H FT-NMR spectrum of rat-liver mitochondrial membranes. The deuterium quadrupole split NMR signal originates from deuterated choline-groups of the phospholipids in the membranes. Temperature 28°C.

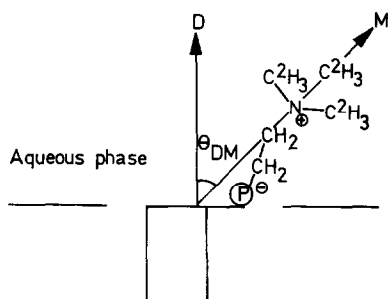


Fig.3. Schematic drawing of the phosphorylcholine group at the surface of the lecithin bilayers. The different coordinate systems used in the text are outlined in the figure, the director (D) and the molecular frame (M). θ_{DM} is the angle between z-axis in director-molecular systems. An isotropic rotation about bonds for methyl groups and the C-N(CH₃)₃-bond has been assumed for the definition of the molecular frame.

with a bilayerstructure. However, the magnitude of the splitting is unexpectedly small compared to other splittings observed for phospholipid model membranes of both synthetic lecithins [8] and extracted lipids as is shown in fig.1. Two apparent explanations, based on the model depicted in fig.3, could be given to the small splitting observed in the membranes. Any explanations have to take into account a change in the angle θ_{DM} (on the average)* compared to the situation existing in the lamellar liquid crystalline system of the pure lipids. Let us consider a case where the choline group moves rapidly so that its motion is 'almost isotropic'. That such a case could occur in the membrane seems, however, less probable since the splitting observed for a liquid crystal, where one would expect more freedom of motion for the choline group, is considerably larger. Therefore we suggest that the choline group in some way is strongly interacting with other molecules in the membrane, probably proteins, which may cause the choline group to be restricted to certain conformations. To investigate, whether such interactions occur, a study of the quadrupole splitting after removal or degradation of the membrane proteins will be performed and reported elsewhere.

In a recent study [8] of the deuteron quadrupole splitting of dimyristoyl lecithin labeled with [Me-²H]choline we found a change in the slope of the tempe-

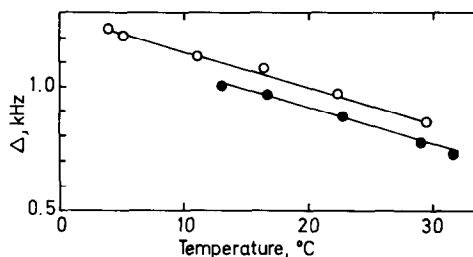


Fig.4. Temperature dependence of the deuteron quadrupole splitting of lamellar liquid crystalline samples of the total lipids of rat liver mitochondria (●) or microsomes (○) labeled with [Me-²H]choline. The water content of the samples was 30% (v/w).

perature-curve at the transition from gel to liquid crystalline state. From fig.4 it may be inferred that for the extracted rat-liver lipids the choline deuteron quadrupole splitting decreases almost linearly with increasing temperature from about 4 to 30°C. It may thus be concluded that the phase transition temperature is below 4°C in this case. This is not surprising, since the lipids of mitochondria and microsomes are known to be very unsaturated. Furthermore, the deuteron quadrupole splitting of dimyristoyl lecithin was found to be approximately 1.5 times as large as that obtained for rat liver lecithin labeled with [Me-²H]choline [12]. Thus, it seems as unsaturation in the acyl chains of the phospholipids causes a change in conformation or the ordering of the choline head group. Further studies of this matter have to be performed before more definite conclusions can be drawn.

Acknowledgement

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* To be exact $\cos^2 \theta_{DM}$, see [10].

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